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13. ABSTRACT (Maximum 200 words)
Much of the first year (01 Jun 91 - 31 May 92) of this project was devoted to developing and refining laboratory techniques for assessing alterations in immune system function, cytogenetics or cytochrome P-450 activity in our principal in situ biomonitor, the cotton rat (Sigmodon hispidus). Small mammal communities were monitored on three uncontaminated control and three heavy metal-petrochemical contaminated sites from January 1991 to March 1992 on the Refinery Waste Site in Cyril, Oklahoma. Seasonal assessments of in situ toxicity were made by returning 10 animals per study site to the laboratory for detailed postmortem examination, including immunotoxic, metabolic, and genotoxic evaluations. Postmortem examinations have revealed significant gross dental lesions in 80% of the rats removed from study sites 3 and 4 (both contaminated). Hepatic total P-450 activity and P-450 O-dealkylation of resorufin ethers have been performed on the first collection of rats from the Cyril site but have not been statistically analyzed. Chromosomes were extracted from bone marrow for mitotic metaphase aberrations, spleen samples were fixed for flow cytometric analysis, and liver biopsies were quick-frozen for later DNA single strand lesion analysis using the alkaline unwinding assay. Preliminary examination of these

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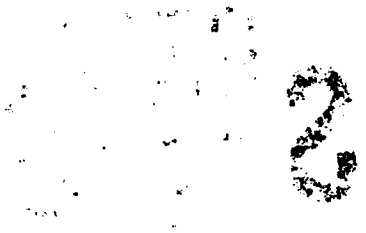
data suggests that spleen cells from animals on petrochemical-contaminated study sites have less nuclear DNA and greater variation in nuclear DNA and greater variation in nuclear DNA content within individuals than those from control sites. We also performed a complete Tier-1 immunological screening panel on each of the 120 animals returned to the laboratory to include lymphoproliferative responsiveness, in vivo cell-mediated response to PHA, natural killer cell activity, macrophage phagocytic activity, and hematology exam. Preliminary analyses indicate significant alterations in lymphoproliferative responsiveness of cultured splenocytes to selected mitogens and interleukin-2, suggesting cell-mediated immune lesions.



ANNUAL TECHNICAL REPORT

Wild Mammalian Biomonitors for Assessing Impacts of Environmental Contamination on Population and Community Ecology.

PROJECT SUMMARY

Much of the first year (01 Jun 91- 31 May 92) of this project was devoted to developing and refining laboratory techniques for assessing alterations in immune system function, cytogenetics or cytochrome P-450 activity in our principal in situ biomonitor, the cotton rat (Sigmodon hispidus). Small mammal communities were monitored on three uncontaminated control and three heavy metal-petrochemical contaminated sites from January 1991 to March 1992 on the Refinery Waste Site in Cyril, Oklahoma. Seasonal assessments of in situ toxicity were made by returning 10 animals per study site to the laboratory for detailed postmortem examination, including immunotoxic, metabolic, and genotoxic evaluations. Postmortem examinations have revealed significant gross dental lesions in 80% of the rats removed from study sites 3 and 4 (both contaminated). Hepatic total P-450 activity and P-450 O-dealkylation of resorufin ethers have been performed on the first collection of rats from the Cyril site but have not been statistically analyzed. Chromosomes were extracted from bone marrow for mitotic metaphase aberrations, spleen samples were fixed for flow cytometric analysis, and liver biopsies were quick-frozen for later DNA single strand lesion analysis using the alkaline unwinding assay. Preliminary examination of these data suggests that spleen cells from animals on petrochemical-contaminated study sites have less nuclear DNA and greater variation in nuclear DNA content within individuals than those from control sites. We also performed a complete Tier-1 immunological screening panel on each of the 120 animals returned to the laboratory to include lymphoproliferative responsiveness, in vivo cell-mediated response to PHA, natural killer cell activity, macrophage phagocytic activity, and hematology exam. Preliminary analyses indicate significant alterations in lymphoproliferative responsiveness of cultured splenocytes to selected mitogens and interleukin-2, suggesting cell-mediated immune lesions.

REPORT PERIOD: 01 Jun 91- 31 May 92

PRINCIPAL INVESTIGATORS:
Dr. Robert L. Lochmniller
Dr. Karen McBee
Dr. Charles A. Qualls
Dr. S. L. (Bud) Burks

OTHER PROJECT PERSONNEL:

Several graduate students and laboratory technicians are both directly and indirectly supported or provide assistance to this project. The following list of individuals have contributed to the project during the first year of funding.

Scott T. McMurry (Ph.D candidate)
Jing Ren Zhang (Ph.D. candidate)
Timothy Propst (M.S. candidate)
Monte L. Thies (Ph.D. candidate)
Barbara C. Bowers (M.S. candidate)
Susan L. Hedinger (M.S. candidate)
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Sundeep Chandra (M.S. candidate)
Madav Parangipe (Ph.D. candidate)
Jim Lish (Laboratory technician)
Kathleen Thies (Research Technician)
Andrea Sampley (Laboratory technician)

RESEARCH OBJECTIVES:

Objective I. To determine the ecological effects and sensitivity of complex mixtures of environmental toxicants at contaminated petrochemical waste sites on the structure and composition of resident populations and communities of small mammals by quantitating:

1. age structure, sex ratio, recruitment, survival of populations.
2. species richness, diversity, similarity of communities.

Objective II. To determine the immunotoxicity, genotoxicity, and metabolic toxicity (physiological response-profiles) of complex mixtures of environmental toxicants in resident small mammals inhabiting contaminated petrochemical waste sites through evaluation of:

1. immune organ development, lymphocyte subpopulations, lymphoproliferation, humoral and cell-mediated immunity.
2. chromosome aberrations, variation in nuclear DNA content, DNA strandbreaks.

3. total and isoenzyme levels of hepatic cytochrome P-450, ultrastructural pathology, free radical concentration.

Objective III. To evaluate the use of enclosed terrestrial mesocosms for conducting both subacute and chronic in situ exposures of cotton rats to complex mixtures of environmental toxicants at contaminated petrochemical waste sites by measuring:

1. reproductive and survival response.
2. physiological response-profiles.

Objective IV. To examine correlations between in situ physiological response-profiles (immunologic, genetic, metabolic endpoints) of a mammalian biomonitor (cotton rat and white-footed mouse) and standard laboratory-derived biological assays for soil leachates from contaminated petrochemical waste sites by using:

1. Ceriodaphnia short-term chronic bioassay.
2. fathead minnow larval survival bioassay.
3. rice seed germination toxicity assay.

STATUS OF RESEARCH:

Study Site: Our research team for this project is currently composed of four tenure-track faculty, two technicians, three doctoral and four masters candidates. Tremendous progress was made over the last year establishing specific study sites and initiating in situ monitoring programs on the Oklahoma Oil Refinery Superfund Site, Cyril, Oklahoma. Three experimental reference or control sites and three contaminated sites were chosen based on their ecological similarity and proximity to the study area. The three contaminated sites include a sludge land-farming area containing heavy metal and petroleum contaminants, a spoil-site adjacent to a series of API-separator ponds containing heavy metal and petroleum contaminants, and a site adjacent to asphalt disposal pits containing a variety of petroleum contaminants. Additionally, we have constructed six mesocosms on the study site to house experimental subjects for intensive physiological monitoring. Three mesocosms are located on the contaminated study sites described above and three control mesocosms were constructed on an area off-site.

Contaminants and Bioassay Results: The primary objective of this project was to evaluate correlation between laboratory derived toxicity response factors of aquatic organisms and plants with community response factors of resident small mammals living in ecosystems contaminated with petrochemical hazardous wastes. As a secondary objective, we are comparing chemical contaminant concentration data from the on-site study areas with EPA recommended Chronic Toxicity Reference Levels specified in the Toxics Characteristics (TC) rules (EPA, March 29, 1990, Fed. Reg., 55(61)11796-11877). The overall approach used in our evaluation was based upon measurement of the response of resident small mammals to deleterious chemicals within the contaminated area as compared with the toxicity test response of selected aquatic organisms, microbial populations, and plants to extracts of soils collected from the study area. Soil samples were collected from each of the six study sites in

conjunction with seasonal trapping efforts and samples returned the laboratory for further analyses.

We used the EPA Toxic Characteristics Leachate Procedure (TCLP) (EPA Method 1311) to prepare extracts of soil samples for chemical analyses and laboratory toxicity assays. We evaluated the potential of using the TCLP extract procedure for conducting laboratory toxicity tests by performing acute toxicity tests with Ceriodaphnia of extracts from inert silica sand, the blank extraction media, and from both contaminated and uncontaminated control sites. The test organisms used in the laboratory toxicity tests were microbes (Photobacterium phosphoreum), water flea (Ceriodaphnia dubia), fathead minnow, (Pimphales promelas), and rice seeds (Oryza sativa).

We spent a large part of the first year evaluating the use of the EPA Toxic Characteristics Leachate Procedure (TCLP) for extraction of soil samples from the Cyril Refinery. The original proposal was to use the TCLP to provide aqueous extracts which could be subsequently analyzed for chemical contaminants as well as performing laboratory toxicity tests with aquatic organisms. The TCLP procedure has not provided a good protocol for obtaining samples for toxicity tests with aquatic organisms because the blank caused too much toxicity to be acceptable. We have returned to using reconstituted water as the extracting fluid. The aqueous extraction procedure imparts no solvent blank toxicity; however, the trade-off is that it may not simulate "worst-case" conditions that could occur in situ.

The aqueous extracts of soil samples collected from control site 1 did not exhibit acutely lethal effects upon Ceriodaphnia after 24 h of exposure. The undiluted extracts from contaminated site 2 were lethal, but there may have been suspended particles interfering with the test; this test will be repeated many more times to verify the results. The aqueous extraction procedure has been used for several sample collection periods with varying degrees of success. The "blank" aqueous extract has exhibited no toxicity, therefore eliminating the problem encountered with the TCLP extraction method. Toxicity of the aqueous extracts to the Ceriodaphnia, Microtox, and rice seed germination tests has indicated that contaminated site 3 contains the highest levels of acutely lethal contaminants. The concentration of toxic metals in samples collected from the area in December 1991 indicated relatively high concentrations of both cadmium and zinc from contaminated site 4 (land farm site). However, the relative toxicity to Ceriodaphnia indicated greater acutely lethal effects from contaminated site 3 (API separator ponds). There was no significant difference in rice seed germination in these soil samples. The dry weight of rice shoot growth was highest in soil samples from contaminated site 4. The laboratory toxicity tests did not seem to correlate with the concentration of toxic metals in the soil.

The most toxic soil sample analyzed to date, was collected from contaminated site 3. This sample had a physical appearance and odor of "oily" materials. The sample aqueous extracts were highly toxic to Microtox, Ceriodaphnia, rice seed germination, and rice shoot growth. Aqueous extracts of this sample were analyzed by a combination of solid phase adsorbent-methanol elution-high performance liquid chromatography, but failed to detect any peaks. The soil sample was directly extracted with methylene chloride, concentrated to 1 ml, and injected onto a gas chromatograph. The chromatogram indicated a cluster of organic compounds which were chromatographically similar to alkyl-aromatics (Fig 1).

Mammalian Population Dynamics: Small mammal population monitoring was initiated in January 1991 and has continued systematically on all six study sites. A total of 2,136 small mammals representing 10 different species has been collected on the study sites during 15,552 trap-nights of sampling. All animals trapped were sexed, aged, general condition noted, reproductive status assessed, and released. Population data have not been analyzed statistically so results are only preliminary. In general, numbers of animals trapped (minimum number known alive) appeared greater in summer on control grids than on contaminated grids (Table 1). Some species were not found or occurred in very low numbers on contaminated study sites, eg. Microtus pinetorum and Blarina brevicauda. All study sites harbored large populations of our primary species of interest, the cotton rat, Sigmodon hispidus). No remarkable differences in community structure were noted as indicated by measures of species richness and diversity (Table 1). Preliminary analysis of community similarity indices indicated that small mammal communities on the control study sites are more similar to each other than they are to the contaminated study sites (Fig. 2), suggesting that contaminants influenced community structure. Recruitment and survival rates are being calculated from these data sets.

Pathology: Pathologic evaluations have been made on cotton rats collected from control and contaminated study sites. We trapped 10 animals per study site once each season and returned animals to the laboratory for detailed pathologic examination. All results, especially of histopathology, have not been summarized. A complete set of tissues is presently being processed from all rats collected from the Cyril site; slides have been prepared from the first collection and the slides from the second collection are currently being processed. However, several notable observations have been made concerning the gross pathology of collected animals. We have noted abnormal coloration of incisor teeth in many cotton rats from contaminated study sites. These lesions appear to be most prominent at grid 4 which represents the land-farm site (Table 2). We have not determined the etiology of these lesions. There is a defect in enamel formation in the abnormal incisors which is associated with the degeneration of ameloblasts. We are having undecalcified sections prepared for histologic examination in the laboratory of Dr. Michael Rohrer, University of Oklahoma School of Dentistry to study the ameloblast/enamel interface. It appears that the lesions are much more prominent in the winter collection than in the summer collection, suggesting soil ingestion may be an important factor associated with the incidence of this pathology.

Urinary bladders of many of the animals have mineralization of the transitional epithelium. We are in the process of evaluating this change which has been noted in animals from the contaminated and reference sites, but appears to be more common in animals from grid 4, the land-farm site.

Basic hematologies were profiled on all animals returned to the laboratory and serum samples have been submitted to a private laboratory for analysis from summer 1991 and winter 1992 collections of animals. Serum will examined for differences in concentration of 19 clinical chemistry parameters, including various enzymes and electrolytes; these results have not yet been received for interpretation.

Our initial results provide strong evidence in support of using both gross and histologic examinations in assessing toxicity in situ using small mammalian residents. These results are only preliminary and further study will be needed to validate their usefulness.

Cytochrome P450 Activity: We have made progress in evaluation of the hepatic cytochrome P450 metabolic profile of cotton rats. We assayed liver tissue samples from cotton rats previously exposed to Aroclor 1254 (A1254) (250 mg/kg) and compared them to cotton rats exposed to 3-methylcholanthrene (3MC) (25 mg/kg) and phenobarbital (PB) (70mg/kg). The results of O-dealkylation of resorufin ethers are expressed as induced divided by control to express results as fold-induction (Fig. 3). Preliminary evaluation of results indicated that the cotton rat behaves similar to domestic "laboratory" rats relative to hepatic cytochrome P450 mediated O-dealkylation of resorufin ethers. These results demonstrate that cotton rats induced with PB specifically increased their metabolism of pentoxyresorufin. Animals induced with 3MC showed a minimal increase in metabolism of pentoxyresorufin. As in the "laboratory" rat, cotton rats had prominent induction of methoxyresorufin, ethoxyresorufin, propoxyresorufin and benzyloxyresorufin. The cotton rat hepatic metabolism of resorufin ethers is somewhat different from the laboratory rat in that it shows the greatest increase in metabolism of propoxyresorufin. Evaluation of animals induced with A1254, a mixed inducer in "laboratory" rats, revealed a similar mixed pattern of induction with increase in metabolism of resorufin ethers that are increased by both 3MC and PB.

An experiment using varying doses of A1254 demonstrated that O-dealkylation of resorufin ethers in the cotton rat is a sensitive indicator of hepatic cytochrome P450 induction. Maximal induction of O-dealkylation of ethoxyresorufin and pentoxyresorufin were achieved at 16 mg/kg A1254 (Fig. 4). In laboratory experiments with "laboratory" rats 500 mg/kg of A1254 is commonly used. In an initial range finding experiment using benzo(a)pyrene we found that it was a mixed-type of cytochrome inducer in the cotton rat. The pattern of hepatic cytochrome induction with benzo(a)pyrene was very similar to that seen with A1254 (Fig. 5).

Evaluation of results of the O-dealkylation of resorufin ethers from animals collected in situ on the Cyril study site are in progress. Initial evaluation of results from our first collection suggest that there is an increase in O-dealkylation of ethoxyresorufin at grid 4, the land-farming site.

Immunotoxicity:

Immunocompetence profiles have been completed on 120 cotton rats collected in situ and returned to the laboratory for analysis during the first year of funding. Assessments of lymphocyte populations in blood and spleen, immune organ development, macrophage activity, complement activity, humoral immunity, and cell-mediated immunity were completed. All assays in our proposed Tier I and II immunocompetence panels are currently functioning with the exception of the T-

cytotoxic assay. We are continuing to modify this assay for use on the cotton rat. In general, we have noted a considerable degree of intraspecific variability in immune competence measures for wild cotton rats.

Statistical analyses of all immunological data have not been completed, so results after the first year of study are merely preliminary. However, our initial analyses do suggest some alterations in immunocompetence of cotton rats as a result of exposure to contaminated study sites. In general, variation about the mean for a number of immune function parameters was greater among animals collected from contaminated study sites compared with those from control sites. This variation suggests some animals in the population are more sensitive to selected contaminants than others; we expect many immune response measures will be bimodal in their distribution on contaminated sites and more normal on control sites. Lymphoproliferative responsiveness of cultured splenocytes to mitogenic stimulation appear to be sensitive to the environmental contaminants on some study sites (Fig. 6). Spontaneous proliferation of splenocytes in non-stimulated cultures was statistically higher in animals from contaminated study sites, especially in summer 1991. Elevated responses were noted for cultures stimulated with concanavalin-A, but depressed responses were noted for those stimulated with interleukin-2 among animals from contaminated study sites (Fig. 7). Treatment effects were also indicated for relative spleen weight (elevated), T-lymphocytes (depressed), and delayed-type hypersensitivity responses (elevated) in animals collected from some contaminated sites (Fig. 7). No differences among study sites were observed for other measures of immunotoxicity. Results from our winter 1992 field collection have not been statistically analyzed.

Genotoxicity:

In September, 1991 and March 1992, 60 cotton rats were returned to the laboratory from the six study sites (10 animals per grid). For each of these animals, chromosomes were extracted from bone marrow for analysis of mitotic metaphase chromosome aberrations, spleens were disaggregated and fixed for flow cytometric analysis, and liver biopsies and thigh skeletal muscle tissues were frozen in liquid nitrogen for subsequent analysis of DNA single strand lesions using the alkaline unwinding assay.

Analysis of 100 metaphase spreads in each of 13 individuals from the September sampling period has been completed. All samples in the genetic analyses are number coded and analyzed in a "blind" manner (i.e. the person conducted the analysis does not know from which grid an animal was collected) and we will not decode these data until all 60 animals from this sampling period have been analyzed. Thus, it would be premature to draw any conclusions from the chromosome aberration analyses at this point, but 59 preparations have yielded scorable karyotypes. Aberrant cells per individual range from zero to four. Summary values for 12 individuals are included in Table 3.

Flow cytometric analysis of all 59 individuals from the September 1991 sampling period has been completed. This includes generation of over 60 internal standard DNA distribution histograms and over 300 DNA distribution histograms from test animals. Spleen cells of animals from the contaminated sites had significantly less nuclear DNA and significantly

greater variation in nuclear DNA content within individuals than animals collected at the uncontaminated localities. Ranges for eight flow cytometric variables are summarized in Table 4. Statistical differences were indicated between individuals from different grids for four of the eight variables. Grids 1 and 6 (controls) were significantly different in the percent of cells in synthesis. Differences in synthesis percentages may reflect slight differences in cell cycle kinetics but is largely uninformative when it occurred with the single pair of grids. All remaining statistically significant differences were for CV G1 and mean G1 and are summarized in Fig 8. Confidence intervals were set around the mean CV G1 for pooled reference site animals. Twenty-five percent of Grid 1, 50 % of Grid 2, 20 % of Grid 3, 50% of Grid 4, none of Grid 5, and 20 % of Grid 6 animals exceeded the upper confidence limits. For pooled sites, 14 % of reference site animals and 40 % of contaminated site animals exceeded the upper confidence limits. These data indicate that animals from the contaminated grids have significantly increased coefficients of variation for mean DNA content indicating the presence of genetic lesions and significantly decreased relative G1 DNA content suggesting that genetic lesions may be resulting in net loss of DNA.

All 60 individuals from the March sampling period also yielded analyzable metaphase chromosome preparations. Photomicroscopic analyses will continue with the September 1991 sample and this sample during the rest of this year. All 60 animals from the March 1992 sampling period have been prepared for flow cytometric analysis. This should be completed by July 1992. Tissues for the Alkaline Unwinding Assay are being stored at -80C and will be analyzed in late 1992 or early 1993. We have begun to purify DNA from brain, skeletal muscle, and liver and should have preliminary data for this assay by the end of August 1992.

Mesocosms:

Six mesocosms were constructed on three contaminated and three control sites to monitor the effects of short and long-term exposure to specific sites of contamination usir g previously characterized cotton rats. A long-term exposure (8-weeks) was recently completed in winter 1991 (February-March). Survival, morphological development, and reproduction were monitored in each mesocosm and surviving animals returned to the laboratory for assessment of immunotoxicity, genotoxicity, and metabolic toxicity as previously outlined for in situ cotton rats. These results have not yet been analyzed.

ESULTING OR ANTICIPATED PUBLICATIONS:

aranjpe, M. G., C. W. Qualls Jr, A. M. Chandra. Altered hepatocellular foci in Cotton Rats (*Sigmodon hispidus*). *Veterinary Pathology* (submitted 1992)

cBee, K., K. Thies, and S. McMurry. Increased DNA content variation in feral rodents as a biomarker of exposure to environmental contaminants. *Environmental Toxicology and Chemistry* (to be submitted).

McMurry, S. T., R. L. Lochmiller, M. R. Vestey, and C. W. Qualls, Jr. Immunological responses of weanling cotton rats (Sigmodon hispidus) to acute benzene and cyclophosphamide exposure. *Environ. Res.* (submitted 1992).

McMurry, S. T., R. L. Lochmiller, M. R. Vestey, and C. W. Qualls, Jr. Influence of protein restriction on immunocompetence of cotton rats (Sigmodon hispidus) exposed to benzene and cyclophosphamide. *Arch. Environ. Contam. Toxic.* (in preparation).

McMurry, S. T., R. L. Lochmiller, M. R. Vestey, and C. W. Qualls, Jr. Immune function in cotton rats (Sigmodon hispidus) following short- and long-term exposure to lead acetate. *Arch. Environ. Contam. Toxic.* (in preparation).

PRESENTATIONS AT MEETINGS:

Lish, J. W., C. W. Qualls Jr, R. L. Lochmiller. Effects of Aroclor 1254 on microsomal O-dealkylation of resorufin ethers in Cotton Rats (*Sigmodon hispidus*). *Society of Environmental Toxicology and Chemistry*, Nov 1992 (abstract submitted).

Paranjpe, M. G., C. W. Qualls Jr, A. M. Chandra. Altered hepatocellular foci in Cotton Rats (*Sigmodon hispidus*). *Society of Environmental Toxicology and Chemistry*, Nov 1992 (abstract submitted).

McBee, K., K. Thies, and S. McMurry. Increased DNA content variation in Sigmodon hispidus at a super fund site. Southwestern Association of Naturalists, Texas Tech University Center at Junction, Junction Texas, 9-11 April 1992.

McBee, K. K. Thies, and S. McMurry. Increased DNA content variation in Sigmodon hispidus at a superfund site. 72 nd Annual Meetings of the American Society of Mammalogists, University of Utah, Salt Lake City Utah, 14-18 June 1992.

McBee, K., K. Thies, and S. McMurry. Increased DNA content variation in feral rodents as a biomarker of exposure to environmental contaminants. Society of Environmental Toxicology and Chemistry 13th Annual Meeting, 8-12 November 1992 (Abstract accepted for presentation).

Burks, S. L., E. Stebler, and A. Sampley. Workshop on use of Microtox NOEC for toxicity reduction evaluations of wastewater samples. Paper presented at S. Central Soc. Environ. Toxicol. Chem., May 1992, Houston, TX.

Helems, R., and S. L. Burks. Use of a physical-chemical chromatography parameter (Kovats Index) to predict acute toxicity of oil refinery effluents. Paper presented at S. Central Soc. Environ. Toxicol. Chem., May 1992, Houston, TX.

McMurry, S. T., M. R. Vestey, R. L. Lochmiller, and C. W. Qualls, Jr. 1991. Effect of in situ environmental contamination on lymphoproliferative response of adult cotton rats to in vitro mitogenic stimulation. Okla. Acad. Sci., Southeast. Okla. State Univ., Durant, OK. Nov. 8.

McMurry, S. T., R. L. Lochmiller, C. W. Qualls, Jr., K. McBee, and S. L. Burks. 1992. Wild cotton rats as bioindicators of environmental contamination: immune system response. Annual Mting, The Southwest. Assoc. Natur., Texas Tech Univ. Center, Junction, TX. April 9-11.

McMurry, S. T. and R. L. Lochmiller. 1992. Assessment of natural killer (NK) cell activity in wild cotton rats (Sigmodon hispidus). 72nd Annual Mting, American Soc. Mammal., Univ. Utah, Salt Lake City, UT. June 14-18.

McMurry, S. T., R. L. Lochmiller, C. W. Qualls, Jr., K. McBee, and S. L. Burks. 1992. Influence of environmental contaminants on population and community structure of resident small mammals. 13th Annual Mting, Soc. Environ. Toxic. Chem., Cincinnati, OH. Nov. 8-12 (abstract accepted).

McMurry, S. T., R. L. Lochmiller, C. W. Qualls, Jr., K. McBee, and S. L. Burks. 1992. Assessing immune system response as an endpoint measurement of in situ exposure to environmental contaminants: a cotton rat model. 13th Annual Mting, Soc. Environ. Toxic. Chem., Cincinnati, OH. Nov. 8-12 (abstract accepted).

Table 1. Estimated mean size, richness, and diversity of small mammal communities inhabiting three control and three contaminated 1-ha trapping grids at the Oklahoma Refining Company Waste Site, Cyril, OK. Data represents the minimum number of animals known alive from January 1991 to August 1991 for control (n=3) and contaminated (n=3) study sites pooled. Results for winter 1992 have not been compiled.

Trap period	Control study sites	Contaminated sites
Jan 1991	51	78
Mar 1991	58	67
May 1991	63	70
June 6-8	55	54
June 27-28	73	42
July 18-20	86	38
Aug 8-11	78	46
Overall		
Species richness	9	9
Diversity	1.14	1.62

Table 2. Gross pathology of upper and lower incisors of cotton rats collected from control and contaminated study sites at the Oklahoma Refinery Company Waste Site, Cyril, OK. Color of incisors was noted as pigmented (orange) or bleached (white).

Grid NO.	Animals examined	No. animals with incisors with 0-100% orange pigment				
		100	75	50	25	0
Upper incisors						
1 (controls)	8*	8	0	0	0	0
5 (controls)	8	8	0	0	0	0
3 (contaminated)	8	6	0	0	0	2
4 (contaminated)	10	2	1	3	2	2
Lower incisors						
1 (controls)	7^	5	2	0	0	0
5 (controls)	7+	7	0	0	0	0
3 (contaminated)	8	4	2	0	0	2
4 (contaminated)	10	0	0	0	2	8

*Rat # SH101 from Grid #1 upper incisors are missing.
^Rat # SH101 and SH133 from Grid #1 lower incisors are missing.
+Rat # SH320 from Grid #5 lower incisors are missing.

Total number of rats examined = 35
Grids #1 and #5 - control
Grids #3 and #4 - contaminated sites

Comment: The normal color of cotton rat incisors is yellow-orange. Of the 16 animals available for examination of upper incisors from two control sites (Grids #1 and #5), all 16 had yellow-orange colored normal teeth; of the 14 animals available for examination of lower incisors from these control sites, 12 had yellow-orange colored normal teeth. Of the 18 rats available for examination of upper incisors from two contaminated sites (Grids #3 and #4), 8 had normal yellow-orange colored teeth and 4 had absolutely white teeth; whereas, the color of teeth was variable, i.e., <100% yellow-orange for the remaining 6 rats. This effect was more pronounced in the examination of lower incisors. Of the 18 rats examined, 10 had absolutely white colored teeth, where only 4 had normal orange colored teeth, and the remaining 4 had <100% orange colored teeth.

Table 3. Chromosome aberration assay summary values for frequency and type of aberrations in cotton rats (n=100 cells) collected from three contaminated and three reference grids. cB=chromatid break, CB=chromosome break, R=ring chromosome, D=dicentric chromosome, TRF=translocation figure, ACF=acentric fragment.

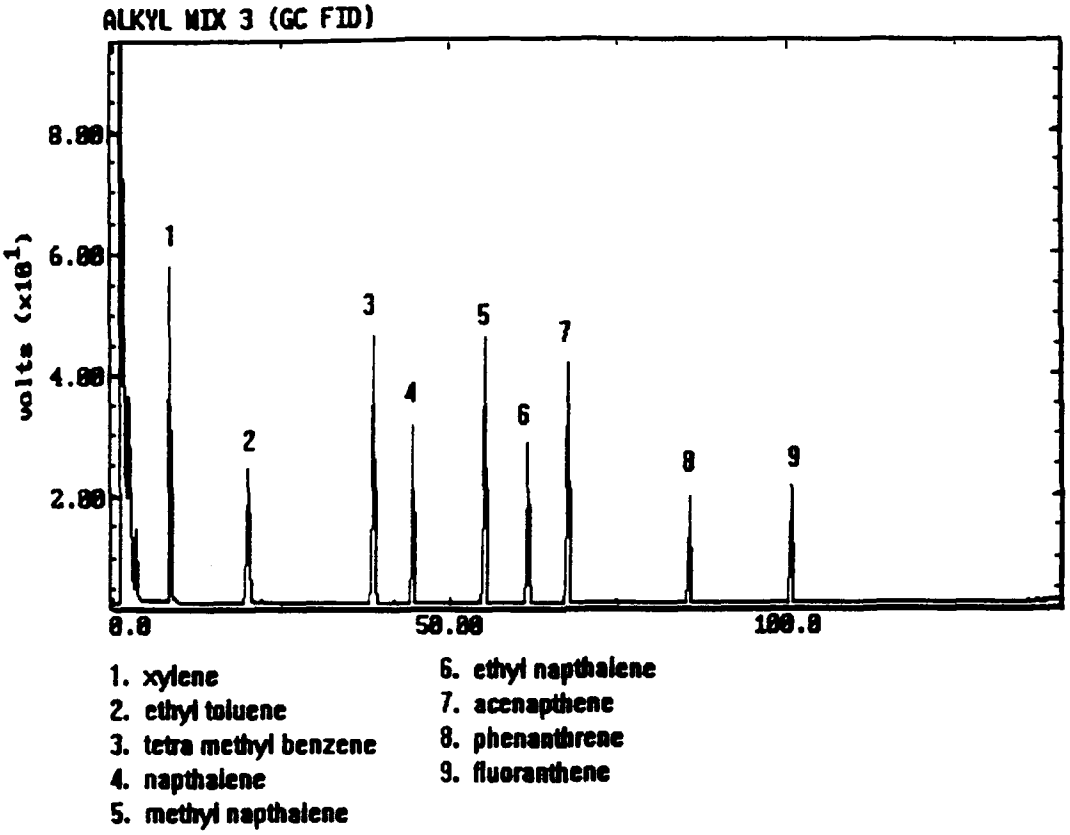
Individual	Grid	cB	CB	R	D	TRF	ACF	Total
OK01076	*	1						1
OK01078	*		3		1			4
OK01079	*							0
OK01080	*							0
OK01081	*	2						2
OK01082	*	2						2
OK01083	*	1						1
OK01084	*	3						3
OK01086	*							0
OK01088	*	2						2
OK01089	*	1						1
OK01095	*	1						1

Table 4. Flow cytometric summary values for coefficient of variation for the G1 peak (CV G1), percent of 20,000 cells in G1 (% G1), coefficient of variation for the G2 peak (CV G2), percent of 20,000 cells in G2 (% G2), percent of 20,000 cells in Synthesis (%S), ratio of number of cells in G2 to number of cells in G1 (G2/G1), percent debris (% Debris), and mean channel (i.e. relative mean DNA content for G1 (Mean G1) from cotton rats collected from three contaminated and three reference sites.

# cells/indiv.	CV G1	% G1	CV G2	% G2
100,000	1.69-1.83	87.87-90.77	1.35-1.44	6.60-8.89

%S	G2/G1	%Debris	Mean G1
2.19-4.45	2.039-2.044	3.98-5.61	62.82-64.83

Figure 1. Gas chromatogram of standard alkyl hydrocarbon mix (top figure) and methylene chloride extract (bottom) of soil sample from enclosure 3.



1. xylene
2. ethyl toluene
3. tetra methyl benzene
4. napthalene
5. methyl napthalene
6. ethyl napthalene
7. acenapthene
8. phenanthrene
9. fluoranthene

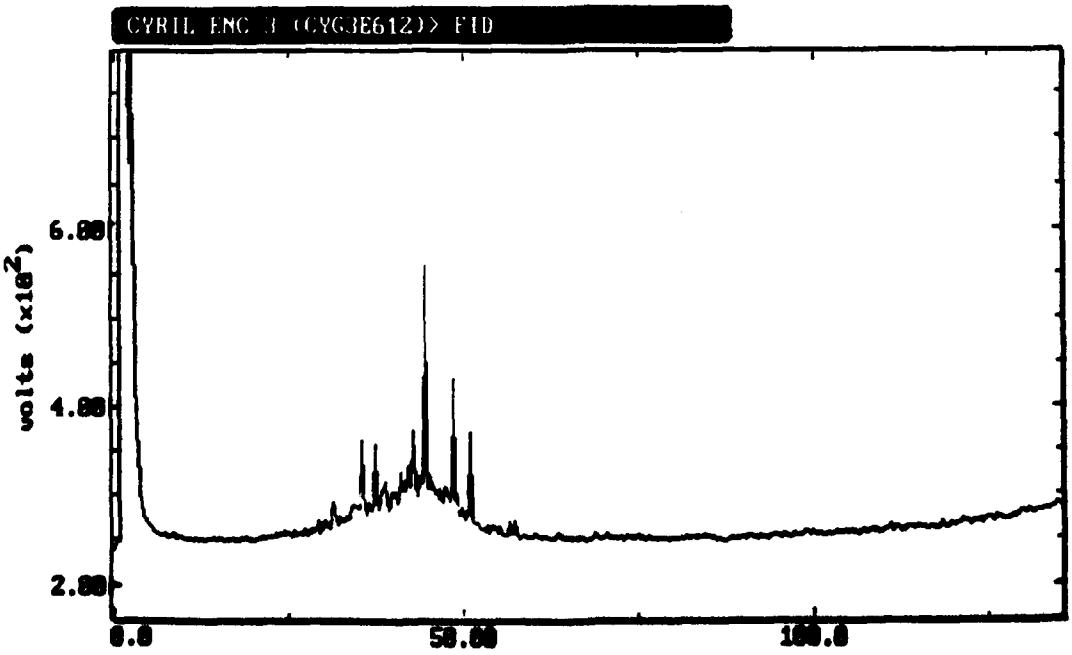
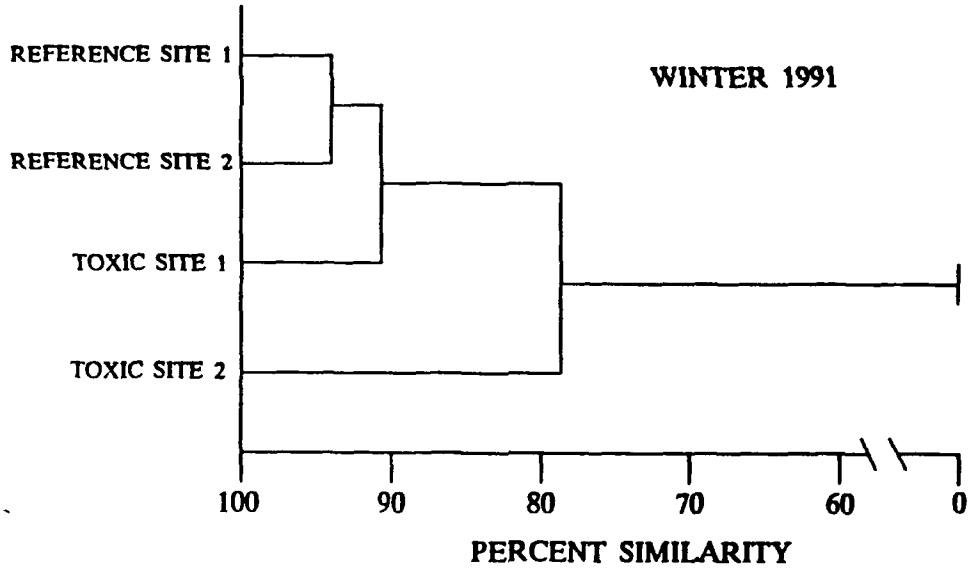


Figure 2. Dendrogram depicting comparisons in the similarity of small mammal communities with respect to species richness and relative abundance of species using Horn's Index. Comparisons were made from results of trapping efforts in winter 1991 and summer 1991 on three reference control sites and three contaminated sites on the Oklahoma Refinery Co. Waste Site, Cyril, OK.



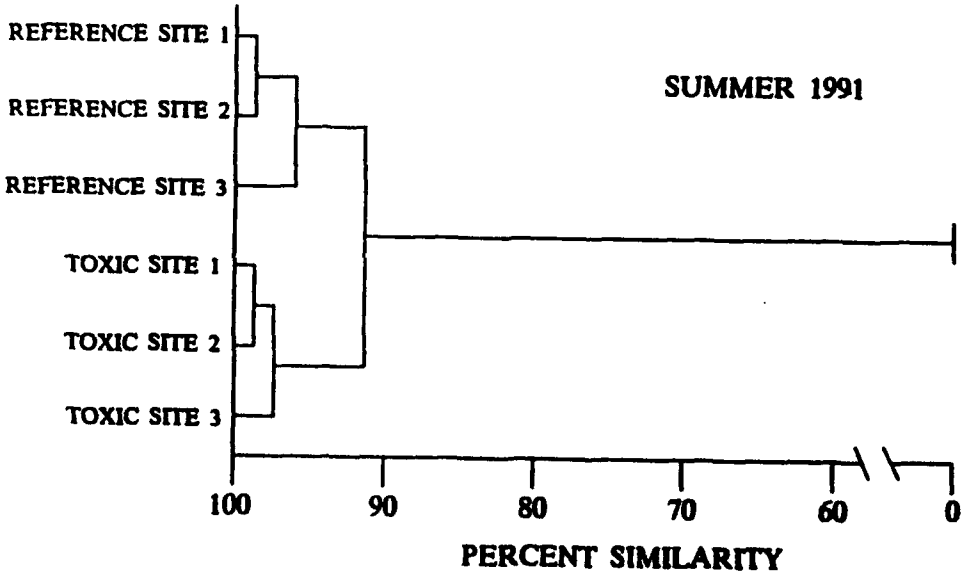


Figure 3. Cytochrome P450 metabolic profiles of cotton rat livers previously exposed to Arclor 1254 (250 mg/kg) compared to rats exposed to 3-methylcholanthrene (3MC, 25 mg/kg) and pentabarbitol (70 mg/kg). Results are expressed as fold-increase in O-dealkylation of the resorufin ethers methoxyresorufin, ethoxyresorufin, propoxyresorufin, pentoxyresorufin, and benzyloxyresorufin.

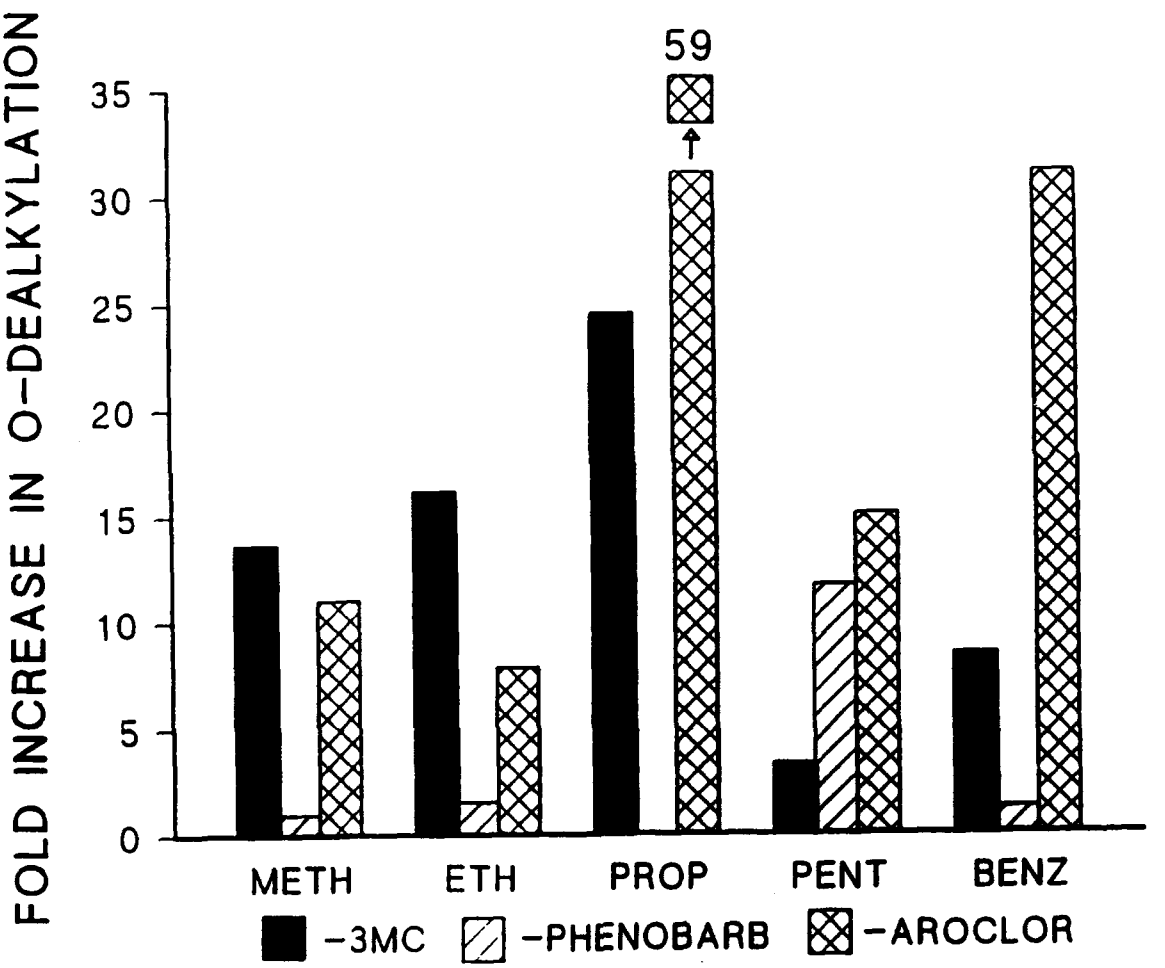


Figure 4. Maximal induction curves (measured as increase in O-dealkylation of the resorufin ethers ethoxyresorufin and pentoxyresorufin) of cotton rats induced with arclor 1254.

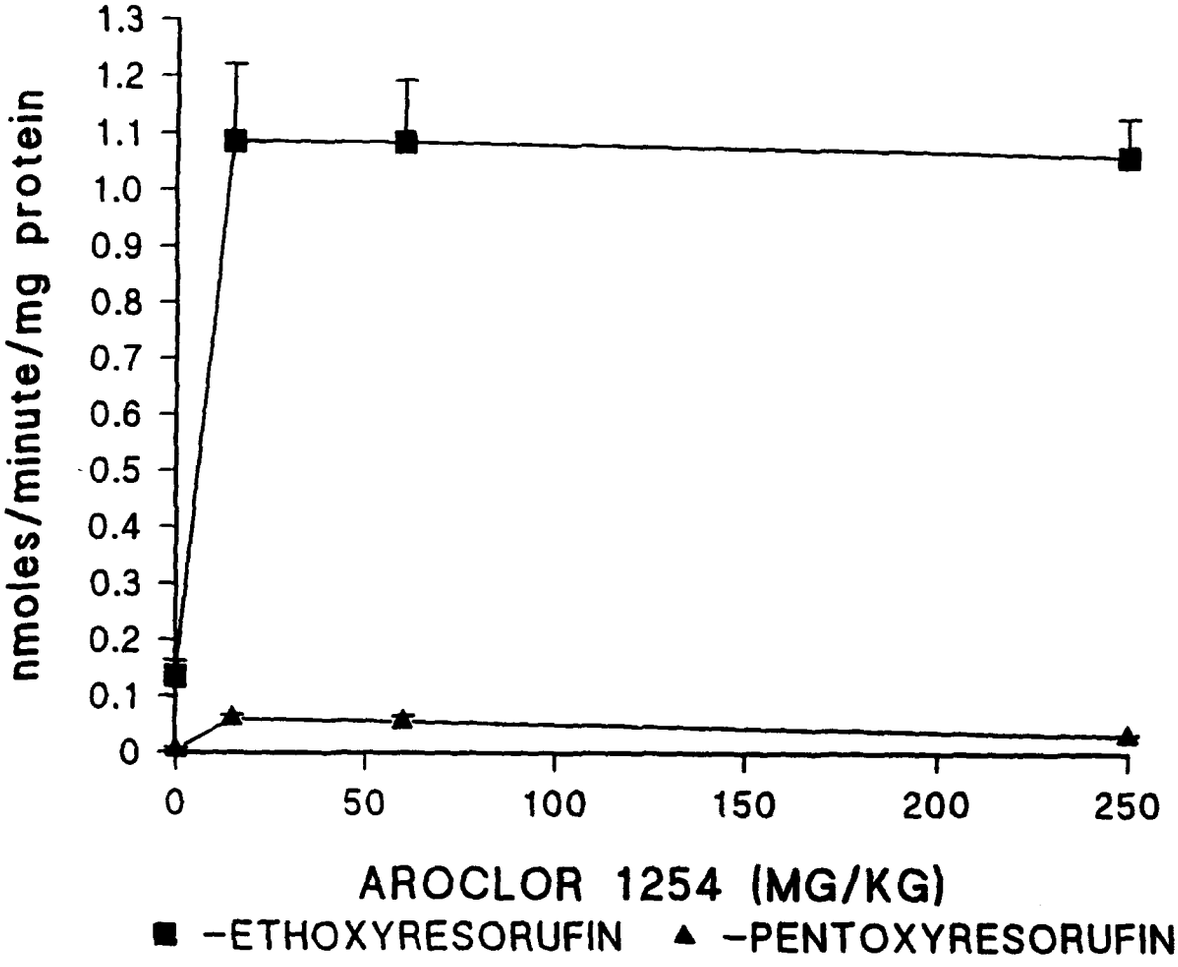


Figure 5. Evaluation of fold-increase in O-dealkylation of the resorufin ethers methoxyresorufin, ethoxyresorufin, propoxyresorufin, pentoxyresorufin, and benzyloxyresorufin in cotton rats induced with benzo(a)pyrene (70 mg/kg).

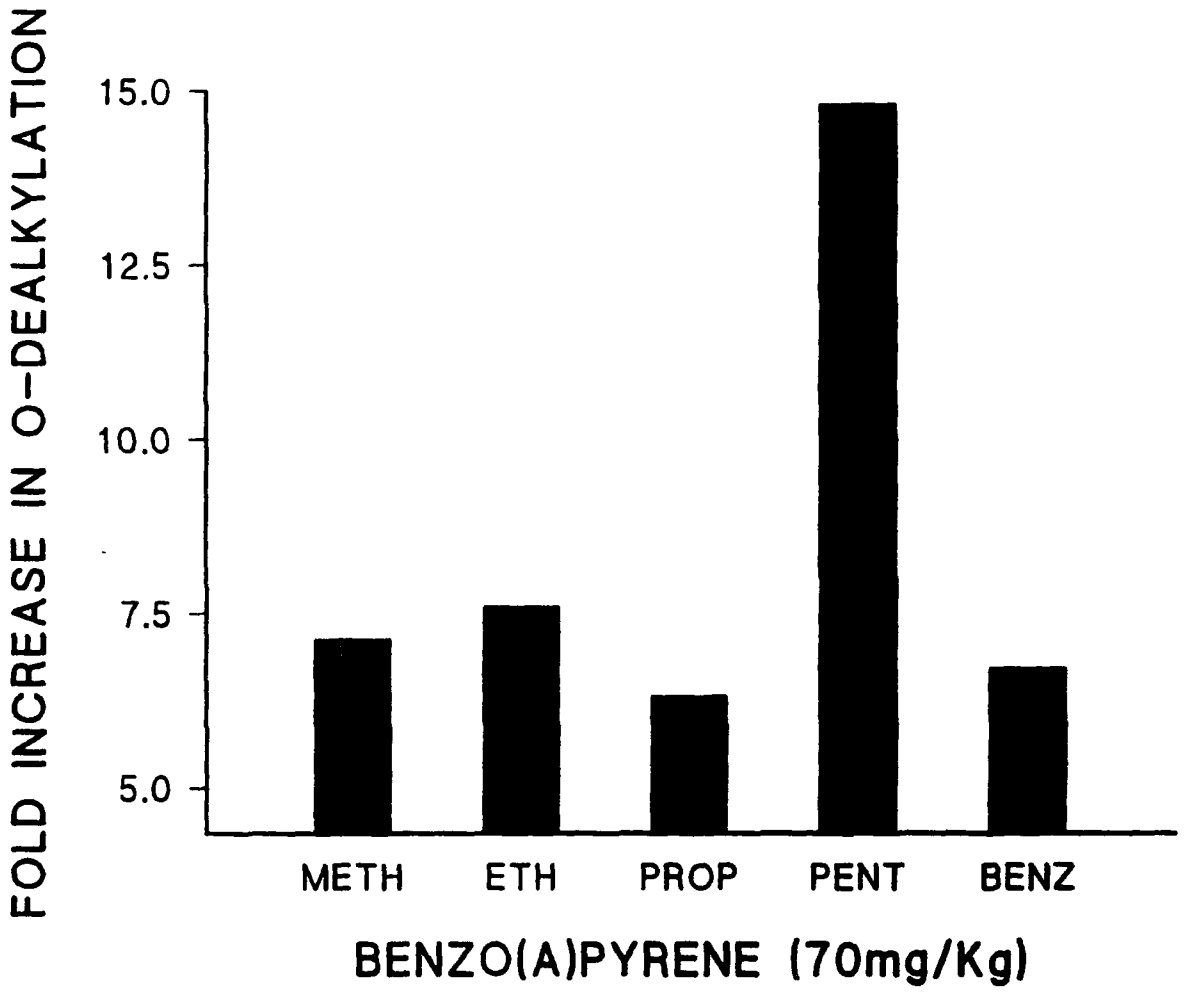


Figure 6. Comparisons of measures of cell-mediated immunity (lymphoproliferative responsiveness of cultured splenocytes to stimulation with Concanavalin A and interleukin-2) among cotton rats collected from three reference control and three contaminated sites on the Oklahoma Refinery Co. Waste Site, Cyril, OK in summer 1991. Values represent means with standard error bars.

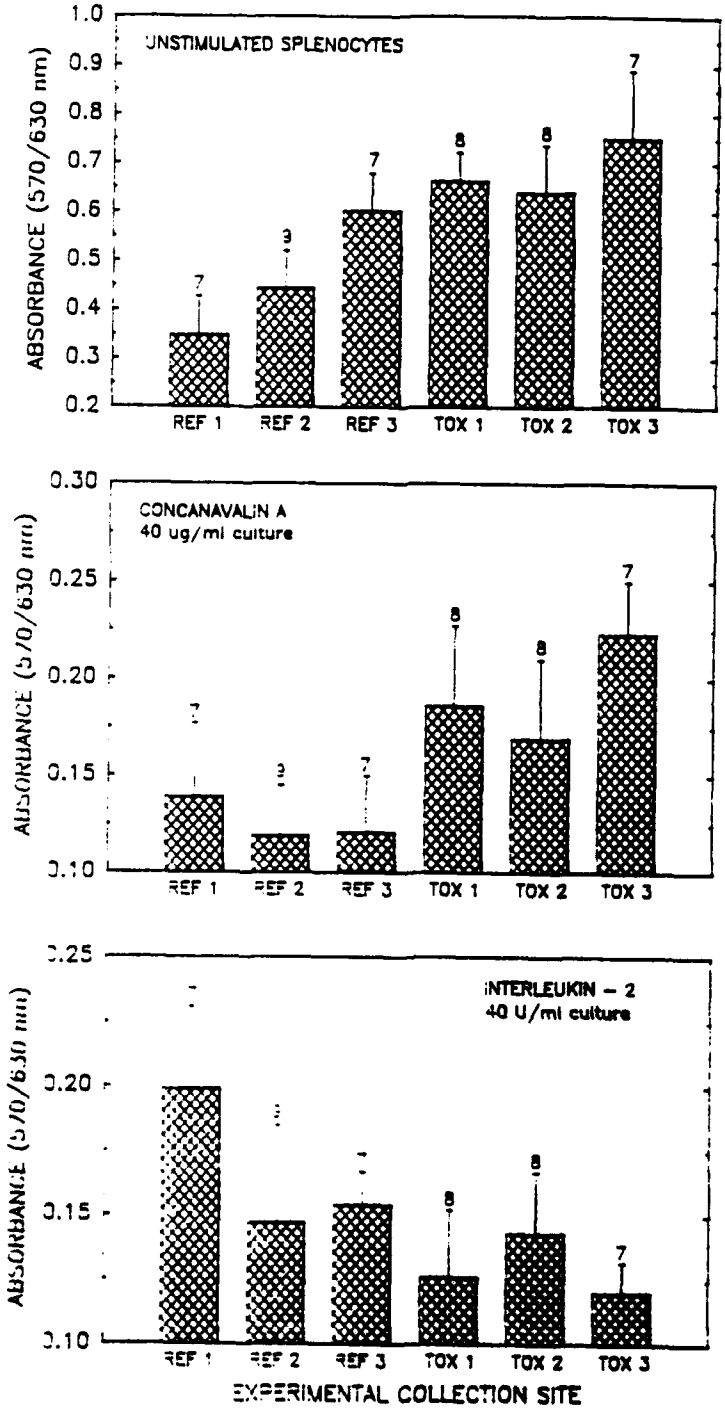


Figure 7. Comparisons of selected measures of immune function in cotton rats collected from three reference control and three contaminated sites on the Oklahoma Refinery Co. Waste Site, Cyril, OK in summer 1991. Values represent means with standard error bars.

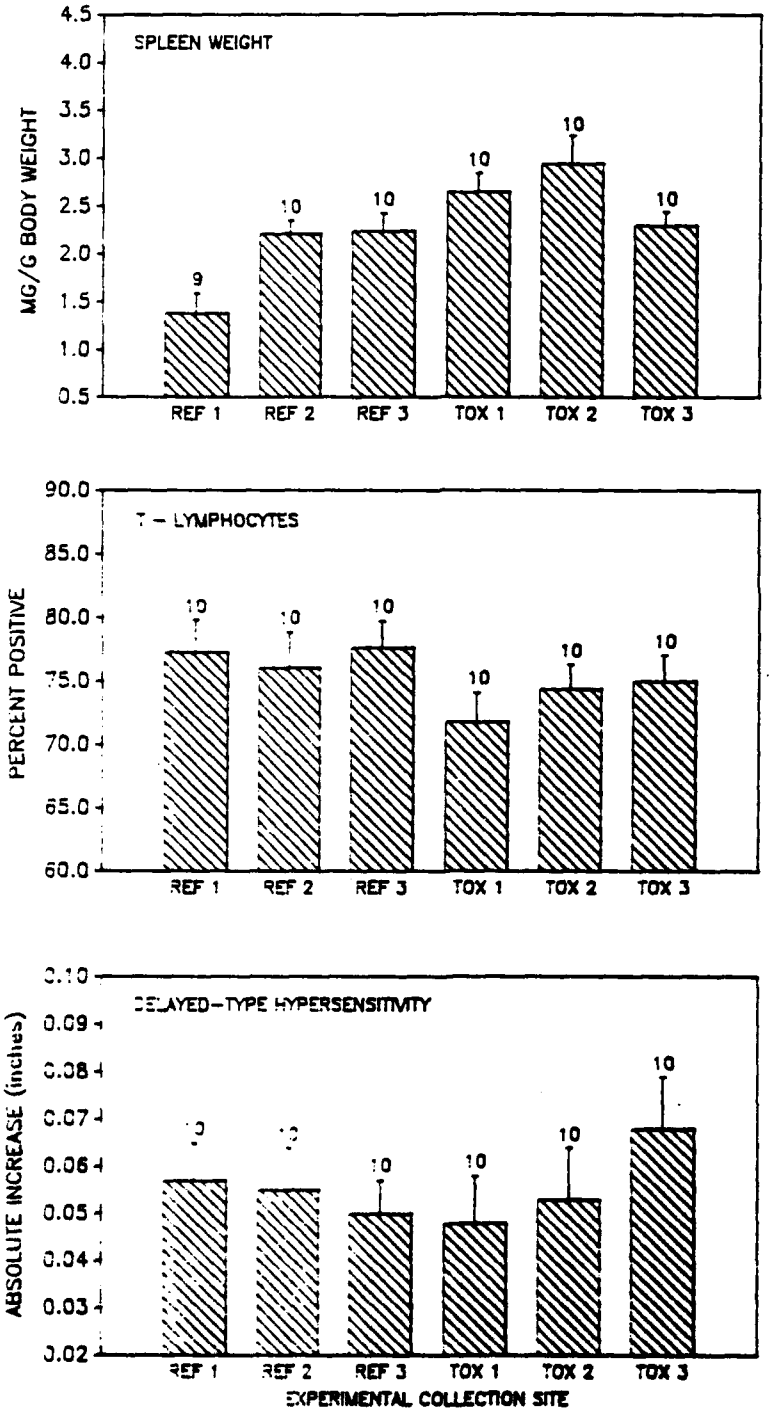


Figure 8. Pair-wise comparisons among all study sites for coefficient of variation for mean DNA content in the G1 peak (above the diagonal) and mean channel of the G1 peak (i.e. relative DNA content) (below the diagonal). * indicates values significantly different at the P=0.05 level with Mann Whitney U test.

Coefficient of Variation - G1

Mean Channel - G1	1	2	3	4	5	6
1	—	*	NS	NS	NS	NS
2	NS	—	NS	NS	*	NS
3	NS	NS	—	NS	NS	NS
4	NS	NS	NS	—	*	NS
5	NS	*	NS	*	—	NS
6	NS	*	*	*	NS	—